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A comprehensive evaluation of skeletal muscle was performed in PG veterans with chronic fatigue and veterans who were deployed but have no medical problems. Using a combination of *in vitro* and *in vivo* measurements we found evidence supporting the hypothesis that muscle function is impaired in veterans with PG illness. Specifically, we found a significant reduction in the *in vivo* mitochondrial ATP producing capacity of Gulf veterans with CF compared to healthy veterans and a high incidence of histological alterations consistent with a mild mitochondrial disorder in all veterans. Veterans with CF also demonstrated a shift in fiber type composition from IIx to IIa. In addition, we found an increased incidence of EMG abnormalities in affected veterans compared to healthy veterans which was most prevalent in veterans with histological evidence of a mitochondrial disorder. MRI measurements showed that there is no difference in the cross-sectional area of the calf muscles of both populations, demonstrating that the decrease in mitochondrial function is not simply the result of severe disuse or deconditioning. Finally, veterans with CF showed a significant decrease in their overall functional ability compared to healthy veterans.

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INTRODUCTION

Since their return from the Persian Gulf region a large number of veterans have reported health problems. Even though no exact count on the prevalence of symptoms and conditions is available the VA Registry shows that about 10% of the 697,000 deployed veterans have reported chronic illnesses with a variety of symptoms, including fatigue, muscle and joint pain, headache, rashes and memory loss. In particular the musculoskeletal system seems to be frequently affected, with as most common symptoms muscle pain, weakness and fatigue. In order to investigate the etiology of the muscle related complaints in the Persian Gulf veterans we performed a comprehensive evaluation of skeletal muscle function in Persian Gulf veterans with severe chronic fatigue and Persian Gulf veterans who were deployed but who have no medical problems. Our primary hypothesis is that muscle function is impaired in Persian Gulf veterans with chronic fatigue. In addition, we hypothesize that the severity of chronic fatigue in this population is related to the degree of muscle dysfunction. To test these hypotheses a battery of tests were performed. Measurements included 31P-magnetic resonance spectroscopy (MRS), magnetic resonance imaging (MRI), histological and biochemical analyses of muscle biopsies, electrodiagnostic evaluation of motor unit recruitment, muscle enzyme assays, isokinetic and isometric testing, and a functional status questionnaire. Complementary to the functional tests, the subjects were screened for AMP deaminase (AMPD) deficiency.

BODY

A. Methods

1) Subjects:

A comprehensive evaluation of skeletal muscle was performed in two subject populations: Persian Gulf veterans with chronic fatigue (PGI) and veterans who were deployed but have no medical problems. Veterans with chronic fatigue are defined as those veterans that report ongoing chronic fatigue, with an onset during or shortly after the war, in combination with muscular complaints. Muscular complaints include muscular pain (severity 3 or more on a scale of 5) and/or muscle weakness (severity 3 or more on a scale of 5). Veterans with chronic fatigue were excluded from this study if they presented any other diagnosable illness. Veterans without medical complaints were recruited to serve as controls in this study. The control subjects were matched with the chronic fatigue veterans with regards to age and sex.

Screening of the subjects:

All subjects were carefully screened at the New Jersey Center for Environmental Hazards Research prior to participation in this study. The screening consisted of a questionnaire, an extensive history, a physical

examination and a psychiatric interview. The screening questionnaire was designed to identify veterans with chronic fatigue. The questionnaire also contained questions related to the use of drugs, alcohol and anabolic steroids, previous medical problems, psychiatric illnesses, hospitalizations, and medication. The physical examination focused on the exclusion of subjects based on other medical conditions that may affect skeletal muscle. Particular attention was paid to lymphadenopathy, peripheral vascular disease, diabetes mellitus and thyroid disease. In addition, all subjects were evaluated for peripheral neuropathy by quantitative testing of the vibration sense. The physical examination was followed by an extensive array of laboratory tests including Complete Blood Count (CBC), electrolytes, renal function, Liver Function Tests, ANA, Rheumatoid factor, Lyme Titer, HIV, and Thyroid function tests with TSH. If the subjects tested positive for any of these tests they were also excluded from this study. In addition, subjects with a history of psychotic disorders or substance abuse (established via the psychiatric diagnostic interview), as well as subjects with a contraindication to an NMR examination were excluded.

A total of 64 Persian Gulf veterans who met the inclusion criteria were identified and agreed to participate in this study. Following initial screening at the New Jersey Center for Environmental Hazards Research the subjects were transported to the General Clinical Research Center (GCRC) at the University of Pennsylvania for a complete evaluation of their lower leg muscles. The subject population consisted of 39 veterans (7 females, 31 males) with chronic fatigue and 25 veterans (5 females and 20 males) without medical complaints. Table 1 provides a summary of the subject's demographics.

	Age	**Height**	**Weight**
Controls	38±2	68.2±0.6	177.7±5.1
PGI	37±1	68.5±0.5	186.2±5.6

Table 1. Characteristics of the healthy control PG veterans and the PG veterans with chronic fatigue (PGI).

2) Functional testing

Functional Status questionnaire: To determine the severity of chronic fatigue in the Persian Gulf veterans we used the functional status questionnaire (FSQ) presented by Jette et al (1). This questionnaire has been shown to be a valid and reliable self-report questionnaire to evaluate physical function in ambulatory patients (1). The Intermediate Activities of Daily Living Subscale provides a cumulative score on 6 questions related to the activity of subjects during the last month. The six questions involve: 1) walking several blocks, 2)walking one block or climbing one flight of stairs, 3) doing work around the house such as cleaning, light yard work or home maintenance, 4) doing errands such as grocery shopping, 5) driving a car or using public transportation and 6) doing vigorous activity such as running, lifting heavy objects or participating in strenuous sports. The lower the score the worse the disability.

Functional Measures: In addition to administering the questionnaire, we assessed the subject's functional status using simple tasks such as walking and stairclimbing. Walking tests consisted of timed walks of 9.1, 15.2, and 30.5 m (30, 50, and 100 ft) without the aid of an assistive device (2). For all distances, subjects were timed at their most comfortable walking speed. The 9.1-m walk was also performed at the *maximum safe speed* (3). In addition to timed walking tests, we measured the time to ascend and descend a flight of stairs (10 steps), without assistance of a handrail or wall. The ability to perform a single-leg heel-rise (plantar flexion onto the ball of the foot) was also tested, as described by Lunsford and Perry (4). Subjects stood facing a wall and were asked to perform as many heel-rises as possible. Subjects were allowed to use the wall only to maintain their balance. The test was discontinued if subjects could not complete a heel-rise through their full, ROM or if they used the wall for assistance, flexed their knee, or asked to stop.

3) Isometric and isokinetic testing

Because many of the Persian Gulf veterans with chronic fatigue report exercise intolerance, prolonged fatigue after exercise and muscle weakness, we quantitatively assessed muscular strength (peak torque) and endurance (fatigue resistance) in the ankle plantar flexors. Both measurements were performed isokinetically and isometrically. In addition, since maximum voluntary contractions rely heavily on the motivation of the subject and his/her ability to recruit and optimally fire all muscle fibers, measurements were also made using electrical stimulation.

Isometric and isokinetic ankle plantar-flexion peak torque was measured on a Biodex Isokinetic Dynamometer. The subjects were seated in upright position on the exercise chair, which was mounted to the floor. Hip flexion angle was set at 90-100 degrees, and the knee position was approximately 0 to 10 degrees of flexion. The axis of the dynamometer was aligned with the lateral malleolus, and the foot was secured to the footplate with a strap placed at the forefoot and ankle. Proximal stabilization was achieved with straps at the chest, hips, and knee. Surface electrodes (bipolar 4x6 inches) were placed over the distal and proximal part of the gastrocnemius.

Isokinetic plantar-flexion torque was measured from the neutral starting position (0° of plantar flexion) through the available plantar-flexion ROM at 60°/s. *Isokinetic peak torque* was defined as the highest torque from a set of 5 maximal reciprocal contractions (5). Subjects performed 2 to 3 submaximal repetitions at increasing intensity as a warm-up. In an effort to optimize the reliability of the testing procedure, each test was repeated up to 2 times if the coefficient of variation (CV) among the 3 highest torques was more than 10%.

Isometric peak torque was assessed at 0 degrees of plantar flexion. Isometric peak torque was defined as the highest torque during 3 contractions (5-second contractions separated by 30 seconds of rest). Similar to the isokinetic test, if the CV among the 3 contractions exceeded 10%, the testing procedure was repeated after a short rest period (up to 2 times). In order to determine the degree of central inactivation a 100 Hz tetanus (supramaximal intensity, 160ms) was superimposed during the voluntary MVCs and the increase in peak torque recorded. The amplitude of the superimposed torque was recorded and expressed as a proportion of the torque measured during unpotentiated tetanic contractions .

Electrically evoked peak torque was determined during a 1ms twitch (100V, biphasic) and a 100 Hz tetanus (supramaximal intensity; 160ms) (6).

Isokinetic endurance was determined during 50 successive maximal contractions of the ankle plantar flexors at a rate of 60°/s (7). *Fatigue* was defined as the relative (%) decrease in work between the first and last thirds of the exercise period. The total work performed during the 50 isokinetic contractions was also recorded.

To discriminate between *central and peripheral fatigue* we implemented a modified version of the fatigue protocol described by Sharma and Miller (8). The subjects performed a sustained MVC for 90 sec. Prior to and during the sustained contraction a tetanic stimuli (100Hz; supramaximal intensity; 160ms duration) was superimposed every 15 sec to verify that the muscle was fully activated. The amplitude of the superimposed torque was determined at each 15s intervals and expressed as a proportion of the torque measured during unpotentiated tetanic contractions (tetanic torque).

4) ^{31}P-Magnetic Resonance spectroscopy (MRS)

In order to assess the *in vivo* metabolic characteristics of skeletal muscle in Persian Gulf veterans with chronic fatigue ^{31}P-Magnetic Resonance Spectroscopy was implemented. In contrast to the muscle biopsies (see below) ^{31}P-MRS is a noninvasive biochemical sampling technique which provides the opportunity to study muscle metabolism in a fully functioning system. All ^{31}P-MRS measurements were performed in a 1 meter, 2.0 Tesla superconducting magnet. The subjects were placed in supine position inside the magnet with their foot on a pedal ergometer operated against variable air pressure (9,10). Spectra were acquired from the medial gastrocnemius using an oblong 4x6cm surface coil, double tuned to both ^{31}P and ^{1}H frequencies . All spectra were acquired with 1,024 data points and 3,000Hz sweep width. Resting spectra were acquired over a period of 9 min, with a pulse repetition time of 30 sec. The *basal content* of the bioenergetically important metabolites inorganic phosphate (Pi), phosphocreatine (PCr) and ADP were measured, assuming an ATP concentration of 8.2mM. The *in vivo oxidative capacity* of skeletal muscle was determined based on the rate of PCr resynthesis following a 12 seconds high repetition, "all-out" exercise protocol and following a steady state

exercise (11,12,13). All exercise and recovery spectra were acquired with a pulse repetition time of 4 sec. The pseudo-first-order rate constant for PCr recovery (k_{PCr}) was determined by least squares fitting of the PCr concentration to a single exponential curve (13). Since maximal rates of glycolysis and ATPase rely on the motivation of the subjects and their ability to optimally recruit all muscle fibers, *ATPase* measurements were performed during voluntary and electrically-induced contractions. The voluntary exercise consisted of a high repetition, "all-out" exercise of 30 sec duration. The stimulation protocol consisted of 1 min of electrically induced contractions using a 100Hz pulse train for 300 msec (supramaximal intensity) at 1 sec intervals. The ATPase flux was determined based on the rate of PCr breakdown during the first 12 sec of voluntary or electrically induced contractions.

5) Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) was used to quantify the maximal cross-sectional area of the calf muscles and to allow calculation of muscle specific force (force/CSA). In addition, the T_2 relaxation properties of skeletal muscle were calculated as an *in vivo* marker of muscle damage.

The maximal muscle cross-sectional area of the medial gastrocnemius, lateral gastrocnemius and soleus was determined using 3D-MRI and an interactive computer segmentation procedure (14). All imaging procedures were performed in a clinical 1.5 Tesla magnet (General Electric), using a birdcage extremity coil. The subjects were placed in supine position with their leg in the coil. 3D data were collected from the mid-thigh to the calcaneus, using a fast gradient-echo sequence, with TR=100 ms, TE=10ms and flip angle of 30°. The images were acquired with an encoding matrix of 256x256x28 and a field of view of 16x16x19.6cm. Chemically selective fat suppression was employed to enhance the definition between muscles. The cross-sectional area of all muscles was determined for each 7mm slice using the interactive computer program, EXTRACTOR, and the correction algorithm previously described (14). Non-muscular regions such as subcutaneus fat, nerves and blood vessels were excluded from the measurements. The maximal cross-sectional area of the medial gastrocnemius, lateral gastrocnemius, and soleus was recorded.

To verify the presence of muscle damage or inflammation in the ankle plantar flexors, T_2 weighted measurements were performed. Multiple T_2-weighted spin-echo images were acquired from the ankle plantar flexor muscles with a TR=2sec, 128x128 matrix, 14x14cm field of view, 2cm slice thickness and TEs of 30, 60 ,90 and 120ms. Pixel by pixel signal intensity was determined as a function of the echo time for regions within the muscle. The characteristic T_2 relaxation rate was calculated based on a single exponential function.

6) Muscle biopsies

Percutaneous biopsies were taken from the right *m.* medial gastrocnemius. Two biopsy samples were acquired: one for qualitative histological evaluation and one for quantitative histochemical analysis (15). Both biopsy samples were mounted in an embedding medium and subsequently frozen in isopentane precooled in liquid nitrogen. Samples were frozen 4 to 6 minutes after excision so as to produce reliable measures for fiber cross sectional area (CSA). The samples were then stored at -70° C until analyzed.

Histological evaluation: Samples were removed from the freezer and placed in a cryostat microtome at -20° C to warm. Serial sections were cut at 10 μm and the sections were stained for hematoxylin and eosin, modified gomori trichrome (16), NADH dehydrogenase (17), ATPase (pH 9.4) (18) and acid phosphatase (19). These stains were used for evaluation of general morphology including variation of muscle fiber size, myofibrillar abnormalities, evidence of necrosis, regeneration, denervation and reinnervation. Mitochondrial abnormalities were judged by alterations in the intermyofibrillar network using the modified gomori trichrome stain and alterations in the NADH dehydrogenase, succinic dehydrogenase (20) and cytochrome oxidase (21) reactions. Glycogen and lipid content were studied with PAS (22) and sudan black (23) reactions.

Histochemical analyses: Samples were removed from the freezer and placed in a cryostat microtome at -20° C to warm. Serial sections of known thickness (6, 10 or 14 μm) were cut and placed onto cover slips for immediate assay by both qualitative and quantitative histochemical procedures. Images of the sections were acquired using a Sony xc 77 CCD camera attached to an Olympus bh-2 microscope linked to a Macintosh Quadra 800 computer. Images were saved and analyzed using NIH image software (written by Wayne Rasband at the US National Institutes of Health, zippy.nimh.nih.gov).

Sections (10 μm) were assayed qualitatively for *myofibrillar adenosine triphosphatase activity* (hATPase) using the techniques of Brooke and Kaiser (24), as modified and done previously (25). Based on their optical density, fibers in the present study were classified as type I, IIa, IIax or IIx. On average, 436 fibers from each sample were used to determine fiber type composition. A minimum of 50 fibers of each type was used for determination of cross-sectional area (CSA). Because the probability existed that each sample would not contain 50 type IIx fibers, their values were combined with those for type IIax fibers for analyses (IIx).

Quantitative histochemical determination of *succinic dehydrogenase activity* (SDH) and *alpha-glycerol phosphate dehydrogenase activity* (GPDH) was used as an estimate of aerobic and anaerobic energy supply, respectively. Enzyme activity was determined by the microdensitiometric technique described by Blanco et al (26) , as done previously (25). Images were captured using the same tools as for hATPase with the exception that a narrow pass interference filter with peak emission of 570 nm was used to assess maximal absorption of

NBT diformazan (NBT-dfz), the in-vitro reaction end product. Enzyme activities were obtained from the difference in optical density between samples incubated in the presence versus the absence of substrate and were expressed as μmol fumerate/l tissue/min and μmol glycerol-3-phosphate/l tissue/min for SDH and GPDH activity, respectively.

Images saved for SDH and GPDH assays were matched to those saved for hATPase, thus allowing values obtained for individual fibers in serial sections to be matched and expressed relative to fiber type and fiber CSA. Hence, for a single fiber of known histochemical type and CSA, both GPDH and SDH activities could be expressed. This provided both a qualitative indicator of fiber properties via hATPase, and quantitative estimates of both anaerobic and aerobic energy supply via GPDH and SDH activities, respectively.

7) Electrodiagnostic evaluation

Muscle dysfunction can be related to a neuropathic as well as a myopathic process. To help in the differentiation between both, needle electromyography and nerve conduction studies were performed as well as electrical stimulation (see above). Compound muscle action potentials were elicited from bilateral tibial and peroneal nerves. F-waves were recorded in the standard fashion at distal stimulation sites and the latencies, amplitudes and conduction velocities were calculated. Monopolar electromyography was performed on the medial gastrocnemius, lateral gastrocnemius and soleus in order to assess denervation, reinnervation and myopathic changes. In addition, the latency and amplitude of the right sural sensory nerve was measured.

8) Genetic screening

As a complementary project to the functional studies performed in these patients, peripheral blood samples were collected for isolation of DNA. The purpose of isolating DNA was to screen for genetic defects, or polymorphisms, which may have predisposed individuals to develop symptoms following military service in the Persian Gulf. Although many genetic defects, or polymorphisms, may be responsible for the pathologic consequences experienced by the Persian Gulf veterans, we only screened for AMP deaminase (AMPD) deficiency.

9) Muscle enzymes

The following biochemical markers of muscle disease/injury were assayed: Serum CPK, LDH and aldolase. Blood samples were drawn prior to any other testing.

B. RESULTS

1) Functional testing

Based on the functional status questionnaire and the functional tasks the overall functional ability of the PGI veterans was significantly lower than that of healthy control veterans (Figs. 1 and 2). The PGI veterans showed an overall functional score (FSQ%) of 73.1±2.4% (range 50.0-100.0%) whereas the healthy veterans scored an average of 98.2±0.7% (range 89.6-100.0%). During the functional tasks the PGI veterans performed between 10 and 30% poorer than the healthy veterans, with the largest difference during stairclimbing. The mean and SEM for the different functional tests is provided in Table 2.

	Healthy	PGI	P	
FSQ (%)	98.2±0.7	73.1±2.4	<0.0001	t=-8.31
100ft walk	19.9±0.4	22.1±0.5	0.0020	t=-3.24
Ascend stairs	3.48±0.15	4.27±0.25	0.017	t=-2.46
Descend stairs	2.91±0.11	3.76±0.27	0.0108	t=-2.64

Table 2. Functional measures in healthy control PG veterans and PG veterans with chronic fatigue (PGI).

2) Isometric and Isokinetic testing

Careful evaluation of muscle strength shows that there is no significant difference is isokinetic or isometric peak torque between healthy Persian Gulf veterans and affected PGI veterans. In addition, during superimposed electrical stimulation only small increases in torque were measured in both subject populations, indicating that both groups performed maximal voluntary contractions. Peak torque measured during electrically-induced contractions also indicated that affected PGI veterans and healthy veterans have the same force producing capacity. Normalizing the isometric peak torque to the total cross-sectional area of the three calf muscles combined showed a specific force (force/CSA) of 2.89±0.17Nm/cm^2 and 2.75±0.10Nm/cm^2, in the affected and healthy veterans respectively. A summary of all force data is provided in Table 3.

	Healthy	PGI	P	t
Isometric PT (ft.lb)	111.44±4.4	100.56±6.2	0.20	t=1.28
Isokinetic PT(ft.lb)	65.38±2.7	62.27±3.0	0.46	t=0.74
Twitch T. (ft.lb)	12.9±0.9	13.7±0.9	0.52	t=0.64
Tetanic T. (ft.lb)	43.1±2.4	37.4±1.3	0.37	t=0.91

Table 3. Voluntary and electrically-induced peak torques in the ankle plantar flexors of healthy control PG veterans and PG veterans with chronic fatigue (PGI).

The relative fatigability during either the isokinetic or isometric fatigue test was not higher in the affected veterans. The relative fatigue during 50 maximal isokinetic contractions was 38.7±2.6% in the healthy veterans and 36.6±3.0% in the affected PGI veterans. Similarly, during the isometric test we measured 49.5±1.8% fatigue in the healthy veterans and 48.3±2.1% in the affected veterans (33.4±9.7%). Of interest to note is that based on the superimposed electrical stimulation the affected veterans tended to have more central inactivation than the healthy veterans with 42.5±% and 33.6±4.8% inactivation, respectively. The total work performed during the isokinetic fatigue test was similar between both populations (627.9±33.6J vs 591.5±30.4J).

3) ^{31}P-Magnetic Resonance spectroscopy (MRS)

MRS data acquired from the medial gastrocnemius of 64 PG veterans showed that the basal phosphate content and intracellular pH of skeletal muscle in Persian Gulf veterans with chronic fatigue is not different from that of healthy PG veterans. However, the PCr-to-Pi ratio tended to be lower in the affected PGI veterans (Fig. 4). Table 4. summarizes the concentration of the different phosphate compounds in both subject populations.

	Healthy	PGI
[PCr] (mM)	39.45±1.07	39.76±0.68
[Pi] (mM)	4.85±0.26	5.37±0.20
PCr/Pi	8.42±0.40	7.73±0.32
pH	7.03±0.01	7.05±0.01

Table 4. Phosphate concentrations and intracellular pH in healthy control PG veterans and PG veterans with chronic fatigue (PGI).

31P-MRS studies performed during recovery following exercise showed that the mitochondrial function of skeletal muscle in Persian Gulf veterans is impaired. The rate of PCr resynthesis, a measure of the *in vivo* oxidative capacity, was significantly slower (P=0.0045) in the Persian Gulf veterans with chronic fatigue than in healthy veterans. The PCr resynthesis rate constant k_{PCr} was 2.38±0.13min^{-1} in the healthy veterans and 1.95±0.08min^{-1} in the affected veterans (Fig. 5). This 20% difference in *in vivo* mitochondrial function between healthy and affected veterans could have important functional consequences.

ATPase measurements during both voluntary and electrically induced contractions showed similar ATPase rates in affected and healthy veterans, demonstrating once again that the affected veterans maximally activated their muscles during voluntary efforts. In both subject populations the ATPase ratesduring voluntary maximal contractions was about 25% higher than during maximal electrically induced contractions (Fig. 6).

4) Magnetic Resonance Imaging (MRI)

The mean maximal cross-sectional area of the calf muscles of healthy veterans and Persian Gulf veterans with chronic fatigue are displayed in Table 6. Note that no difference was observed in the CSA of both subject populations, indicating little or no disuse atrophy in the affected Persian Gulf veterans. The total maximal CSA was 53.5±1.9cm^2 and 49.0±1.7cm^2 in the healthy and sick veterans with chronic fatigue, respectively.

	Lateral Gastrocnemius	Medial Gastrocnemius	Soleus	Total CSA
Control	10.4±0.4	15.4±10.6	25.9±0.8	53.5±1.9
PGI	9.2±0.4	14.6±0.5	24.6±0.8	49.0±1.7

Table 6: Cross-sectional area (CSA) of the three calf muscles in healthy and affected Persian Gulf veterans with chronic fatigue (PGI).

T_2 weighted measurements performed on the three calf muscles of healthy and affected veterans showed no difference in the mean T_2 values of both populations. Also no significant difference was found in the T_2 relaxation rates of the three ankle plantar flexor muscles (Table 7).

	Lateral Gastrocnemius	Medial Gastrocnemius	Soleus	Total CSA
Control (ms)	28.44±0.65	28.28±0.85	29.33±0.84	53.5±1.9
PGI (ms)	27.48±0.53	26.44±0.59	28.44±0.56	49.0±1.7

Table 7: T_2 MR relaxation rates in the three calf muscles of healthy and affected Persian Gulf veterans with chronic fatigue (PGI).

5) Muscle biopsies

Quantitative histochemistry: As expected, the morphological/histochemical characteristics of muscle fibers differed among fibers types in both subject populations. Overall, there was a hierarchy for fiber type percentage (I > IIa > IIx, $p \leq 0.0001$) and for fiber cross-sectional area (CSA) (IIa > IIx > I, $p \leq 0.0001$). (Table 8). A comparison between the two veterans populations showed that the affected veterans had a smaller percentage of type IIx fibers (11.3±2.4% vs 18.7±3.3%) compared to the healthy veterans (Fig. 7). Affected veterans showed a shift in the fiber type composition from IIx to IIa, whereas the number of type I fibers remained constant.

	I	63.3 ± 5.1	6,474 ± 394	59.6 ± 5.4
Healthy	**IIa**	18.8 ± 4.1	7,929 ± 502	21.7 ± 3.6
	IIx	17.1 ± 3.6	7,498 ± 750	18.7 ± 3.3
	I	63.8 ± 3.0	6,591 ± 551	61.7 ± 3.1
PGI	**IIa**	25.1 ± 1.9	7,328 ± 903	27.0 ± 1.8
	IIx	11.1 ± 2.4	6,965± 1,118	11.3 ± 2.4

Table 8: Fiber type specific morphology of the medial gastrocnemus in healthy and affected (PGI) veterans. FT, fiber type I, IIa or IIx. FT%, fiber type percentage. CSA, cross-sectional area in μm^2. %CSA, relative area of muscle occupied by given fiber type.

With respect to enzymes of energy supply, a fiber type effect for SDH activity was shown with type I > IIa > IIx ($p \leq 0.0001$) (Table 9). GPD activity also showed a fiber type effect with type IIx > IIa > I ($p \leq 0.0001$), but no effect for group (Table 9). In an effort to present a more functional description of the fatigue characteristics of the muscle and its fibers, the fiber type specific enzyme activity was multiplied by the relative area occupied by each fiber type. Both %CSA*SDH and %CSA*GPDH showed a fiber type effect as well as group effect ($P<0.05$) (Figs 8 and 9; table 10). A comparsion between subject groups showed that the SDH*%CSA for the type IIx fibers is 2.2 fold higher in the healthy veterans compared to the affected veterans (Fig. 8). Similarly, GPD*%CSA in type IIx fibers of healthy veterans was 3-fold higher than that of affected veterans (Fig. 9).

		Type I	**Type IIa**	**Type IIax**
GPD (mmol/l/min)				
	Control	0.04±0.01	0.09 ±0.01	0.13±0.02
	PGI	0.05±0.01	0.11±0.02	0.13±0.02
SDH (mmol/l/min)				
	Control	0.86±0.13	0.74±0.11	0.80±0.19
	PGI	0.93±0.13	0.77±0.10	0.66±0.09
SDH/GPD				
	Control	16.7±2.9	6.3±1.1	3.9±0.6
	PGI	16.2±1.6	7.1±1.1	5.3±0.8

Table 9: Fiber type specific enzyme actvity in the medial gastrocnemus of healthy and affected (PGI) veterans.

		%CSA* GPD	%CSA*SDH
Healthy	I	871 ± 127	12,802 ± 1,735
	IIa	765 ± 203	4,773 ± 1,681
	IIx	1,246 ± 397	3,845 ± 1,213
PGI	I	1,026 ± 3.0	15,640 ± 2,329
	IIa	904 ± 196	5,431 ± 956
	IIx	414 ± 128	1,710± 451

Table 10: Fiber type specific %CSA*SDH and %CSA*GPD measured in the medial gastrocnemiusof healthy and affected PG veterans (PGI).

Qualitative histology: Careful evaluation of muscle biopsies acquired from the medial gastrocnemius showed a 29% incidence of nonspecific abnormalities in the Persian Gulf veterans. The most common variation was increased subsarcolemmal staining for SDH (31.2% incidence), trichrome (42%) and cytochrome oxidase (24.3%), indicating the presence of a mild mitochondrial disorders. Other non specific alterations included increased phosphatase staining, increased variation in fiber size, a mild increase in central nuclei and the occasional presence of angular fibers. However, a comparison between healthy veterans and affected veterans did not show a difference between both populations. Also no ragged fibers were observed in either subject group.

6) Blood results

No difference was found between sick and healthy veterans based on CPK, aldolase or LDH (Table 11).

	Alodolase	CPK	LDH
Control n=25	4.62±0.4	151±20	420±28
sick n=34	5.4±0.6	158±23	440±17

Table 11. Muscle enzyme activity in healthy control veterans and veterans with chronic fatigue.

7) Electrodiagnostic evaluation

The results of the *nerve conduction* study are given in Table 12.. No difference was found in the latencies, amplitudes and conduction velocities of the tibial and peroneal nerve of healthy and affected PG veterans, indicating the absence of a potential peripheral neuropathy in our veteran population. Note that all veterans were screened for a potential neuropathy based on vibration sense prior to their participation in the study.

Tibial Nerve

	Conduction velocity (m/s)	F wave	Amplitude Distal (mV)	Amplitude proximal (mV)	latency proximal (ms)	latency distal (ms)
Control	50.0±0.7	50.1±0.5	10.7±0.6	10.0±0.6	13.1±0.2	5.0±0.1
Sick	50.9±0.5	50.0±0.5	11.0±0.6	10.6±0.6	12.8±0.2	4.9±0.1

Peroneal Nerve

	Conduction velocity (m/s)	F wave	Amplitude Distal (mV)	Amplitude proximal (mV)	latency proximal (ms)	latency distal (ms)
Control	47.3± 0.5	50.5±0.6	5.1±0.3	4.9±0.3	11.1±0.2	5.0±0.2
Sick	48.0±0.6	49.6±0.5	5.4 ±0.3	5.0±0.3	11.1±0.2	4.5±0.1

Sural Nerve

	Amplitude	Latency
Control	24.0±1.2	3.6±0.1
Sick	24.1±1.1	3.6±0.1

Monopolar electromyography showed an increased incidence ($P<0.01$) of neurogenic abnormalities in the affected veterans. While no abnormalities (0% incidence) were found in any of the healthy veterans the overall incidence of a neurogenic abnormality in the affected veterans was 24%. 6/34 subjects had abnormal motor unit morphology with increased numbers of polyphasic potentials of increased amplitude and duration, indicative of

chronic denervation/reinnervation. 9/34 subjects showed decreased interference patterns during maximal contraction, of which 4 had an increased firing rate, indicative of chronic lower motor unit abnormalities. Finally, one of the subjects showed spontaneous activity at rest and increased insertional activity, an indicator of either acute denervation or muscle membrane instability.

8) Genetic screening

AMPD1 genotype was determined in 25 healthy veterans and 33 affected veterans by use of a polymerase chain reaction-based, allele-specific oligonucleotide detection assay. In the affected group 30 subjects were homozygous for the normal sequence at nucleotide 34 in his/her AMPD1 gene, 3 were heterozygous. Similarly, in the healthy group 21 subjects were homozygous and 4 heterozygous.

9) Regression analysis

Logistic regression indicates that the FSQ% is very different between the healthy veterans and affected veterans. In fact if the FSQ% is put in the model first, then it explains so much variance that nothing beyond it is significant. However, if it is entered in the model last, then the time to ascend 10 steps ($p < 0.01$), the frequency of motor unit abnormalities on EMG during maximal effort ($p < 0.01$) and the *in vivo* mitochondrial capacity kPCr ($p < 0.05$) are significant predictors of whether the veterans are affected or not. Even taking into account these variables, the FSQ% still predicted whether the veterans were affected or not ($p < 0.001$). The affected veterans took more time to ascend 10 steps (4.27 +/- 0.25 sec vs 3.48 +/- 0.15;), they had more EMG abnormalities (35% vs 0%), they had a lower PCr resynthesis rate (1.94 +/- 0.08 vs 2.38 +/- 0.12) and finally they had lower FSQ% scores (98.2 +/- 0.7% vs 73.1 +/- 2.4%). All these factors stayed significant after age and force/CSA were entered into the model first.

The next set of conclusions is based on potential interactions among variables. There was a positive correlation between the distal amplitudes from the two nerves (peroneal and tibial) ($r = + 0.48$) and the proximal amplitudes ($r = + 0.49$); the resulting interactions also predicted who was affected ($p's < 0.05$). For both measurements, there was a good relationship between the two variables in affected veterans ($p's < 0.01$), but no relationship in healthy veterans. Thus, when the two variables were considered together, they were better predictors of who was affected than either variable alone and the combination was significant ($p's < 0.01$).

There was also an interaction between SDH and GPD in the type IIx muscle fibers in predicting who was affected ($p < 0.01$). There was a significant positive relationship between these two variables in healthy subjects ($r = + 0.93$, $p < 0.001$), but only a marginally significant relationship in affected subjects ($r = + 0.62$, $p = 0.54$). The interaction probably comes from the fact that the slope of this relationship is greater for

units for each unit change in SDH) ($p < 0.05$). Thus, there is likely an abnormality in the balance between glycolytic and oxidative functions in the type IIx fibers in affected subjects.

Veterans with a mitochondrial abnormality on biopsy evaluation had a higher frequency of EMG abnormalities (31%) than those without abnormalities (9%) (Fisher test, $p < 0.05$). Those with mitochondrial pathology also had lower PCr/Pi ratios (7.22 +/- 0.48) than those without pathology (8.63 +/- 0.31) ($p < 0.05$), even if differences in group (healthy vs affected) and age were adjusted for.

A comparison between subjects with a large degree (>5% augmentation) of central inactivation during MVC and a low degree (<5% augmentation) of central inactivation showed that subjects with a lot of augmentation also demonstrate a greater amount of central fatigue (0.50+/- 0.06 vs 0.28 +/- 0.04; $p < 0.01$). In addition, subjects with a high degree of augmentation during MVC showed slower 30 ft max walk times (5.50 +/- 0.21 sec) than those with little augmentation (4.84 +/- 0.12 sec) ($p < 0.01$), and they took more time to climb 10 steps (4.33 +/- 0.30 vs 3.60 +/- 0.13) ($p <0.05$).

%CSA-SDH in Type IIx fibers predicted in a negative way the time required to ascend 10 steps, even after group differences were removed ($p <0.05$). That is longer times were associated with lower SDH levels. In addition, higher levels of %CSA-GDP in type IIx fibers were associated with higher %CSA-SDH in the same muscle type after the affect of group was removed ($p < 0.01$).

Finally, the presence of EMG abnormalities during max contraction predicted in a negative way the FSQ% after group differences were removed ($p < 0.05$). That is EMG abnormalities were associated with lower %FSQ scores.

KEY RESEARCH ACCOMPLISHMENTS

The following important findings were made:

- Performance on functional tests showed a significant reduction in the overall functional ability of veterans with chronic fatigue, with an FSQ of 98.2±0.7% vs 73.1±2.4%.

- The most important finding in this study was a 20% decrease in the *in vivo* mitochondrial ATP producing capacity of veterans with chronic fatigue compared to healthy veterans.

- MRI measurements acquired of both populations showed that there is no difference in the cross-sectional area of the calf muscles of both populations, indicating that the decrease in mitochondrial function in veterans with chronic fatigue is not simply due to a severe degree of disuse.

- Quantitative histochemical analysis of muscle biopsies showed a shift in the muscle fiber type composition of affected veterans from IIx to IIa. In addition, SDH*%CSA and GPD*%CSA for type IIx fibers was 2-3 fold lower in the affected veterans versus the healthy veterans.

- There was a high incidence (29%) of nonspecific histological abnormalities in all veterans (healthy and affected). The most common histological variation was an increased subsarcolemmal staining for oxidative enzymes which is consistent with the presence of a mild mitochondrial disorder.

- Veterans with a mild mitochondrial disorder also demonstrated an increased incidence of EMG abnormalities.

- No difference was found in ankle plantar flexor muscular strength and fatigue resistance of Persian Gulf veterans with chronic fatigue and healthy control veterans.

REPORTABLE OUTCOMES

ABSTRACTS:

Lacey, S., Robinson, K., Umille, E., Plotkin, R., Vandenborne, K., D'Esposito, M., DeLuca, J., Natelson, B.: Evaluation of working memory in Persian Gulf War veterans with chronic fatigue. (Federally Sponsored Gulf War Veterans' Illnesses Research, Arlington, Virginia, June, 1999)

Vandenborne, K., Walter, G., Lenrow, D., Leigh, J.S., Fishman, A.P.: Skeletal muscle in Persian Gulf veterans. *J Chronic Fatigue Syndrome* 2(2/3): 137-138, 1996.

MANUSCRIPTS:

Lacey, S., Umille, E., Plotkin, R., DeLuca, J., D'Esposito, M., Natelson, B., Ottenweller, J., Vandenborne, K., Robinson, K.: Working memory in Persian Gulf War veterans with chronic fatigue, *Am. J. Neuropsych*, (in preparation)

Vandenborne K., Eastlack, M., Leigh, J.S., Walter, G., Swift, A., Natelson, B., Ottenweller, J.: Mitochondrial Function in Persian Gulf War veterans with chronic fatigue (in preparation).

Shotland, D., Lenrow, D., Eastlack, M., Gibbs, J., Natelson, B., Ottenweller, J.: EMG and histological evaulation in Persian Gulf War veterans with chronic fatigue (in preparation).

FUNDING:

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CONCLUSIONS

In an effort to investigate the etiology responsible for the ongoing chronic fatigue and muscle weakness in veterans with Persian Gulf illness we performed a comprehensive evaluation of skeletal muscle in PG veterans with chronic fatigue (n=34) and veterans who were deployed but have no medial problems (n=25). Measurements included 31P-magnetic resonance spectroscopy (MRS), magnetic resonance imaging (MRI), histological and quantitative histochemical analyses of muscle biopsies, electrodiagnostic evaluation of motor unit recruitment, muscle enzyme assays, isokinetic and isometric testing, and functional measures. Our data strongly support the hypothesis that muscular abnormalities contribute to the symptom profile of Persian Gulf veterans. Performance on functional tests showed a significant reduction in the overall functional ability of veterans with chronic fatigue, with an FSQ of 98.2±0.7% vs 73.1±2.4%. The most important finding in this study was however a 20% decrease in the *in vivo* mitochondrial ATP producing capacity of veterans with chronic fatigue compared to healthy veterans as well as an increased incidence of EMG abnormalities. MRI measurements acquired of both populations showed that there is no difference in the cross-sectional area of the calf muscles of both populations, indicating that the decrease in mitochondrial function in veterans with chronic fatigue is not simply due to a severe degree of disuse. Quantitative histochemical analysis of muscle biopsies showed a shift in the muscle fiber type composition of affected veterans from IIx to IIa. In addition, SDH*%CSA and GPD*%CSA for type IIx fibers was 2-3 fold lower in the affected veterans versus the healthy veterans. As such the *in vitro* muscle data support the observed reduction in the *in vivo* mitochondrial function following "all-out" maximal exercise. Of interest was also the observed high incidence (29%) of nonspecific abnormalities in all veterans (healthy and affected) during histological evaluation. The most common histological variation was an increased subsarcolemmal staining for oxidative enzymes which is consistent with the presence of a mild mitochondrial disorder. Veterans with a mild mitochondrial disorder also demonstrated an increased incidence of EMG abnormalities. Finally, isokinetic and isometric testing of the plantar flexors showed no difference between the muscular strength of Persian Gulf veterans with chronic fatigue and healthy control veterans. In addition, the relative fatigability was not higher in the veterans with chronic fatigue and standard clinical bloodwork showed no difference in CPK, aldolase and LDH between healthy control veterans and veterans with chronic fatigue.

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APPENDICES

Functional Assessment

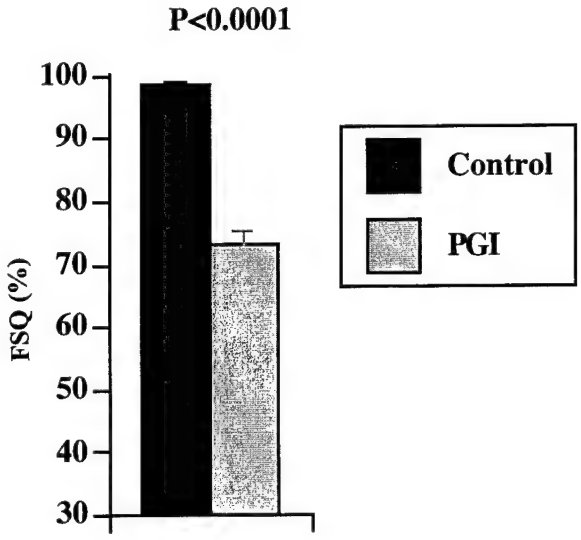

Fig. 1 Results of the functional status questionnaire (FSQ) in the healthy control veterans and the veterans with chronic fatigue (PGI).

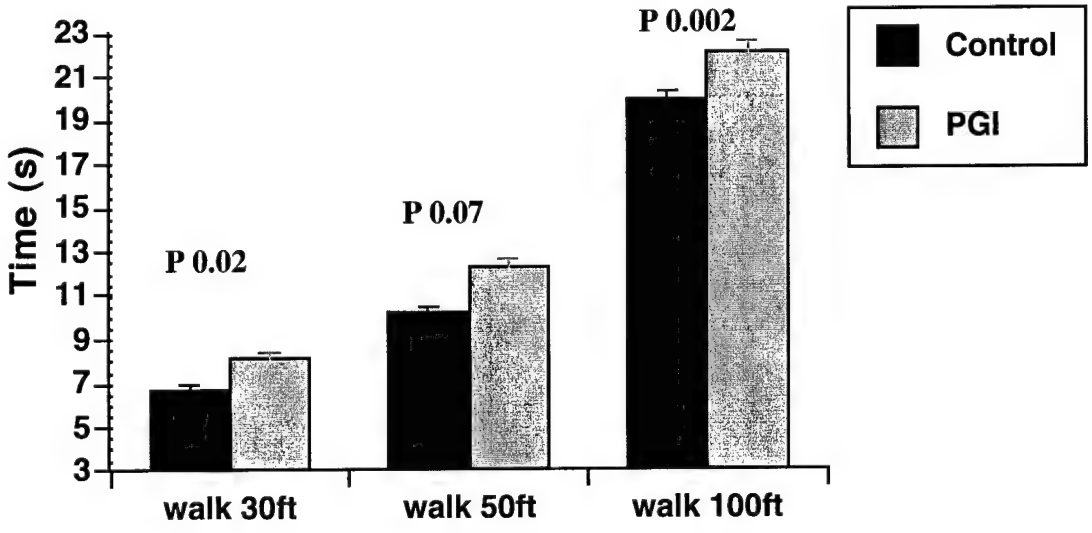

Fig. 2 Results of the timed walking tests in the healthy control veterans and the veterans with chronic fatigue (PGI).

Functional Assessment

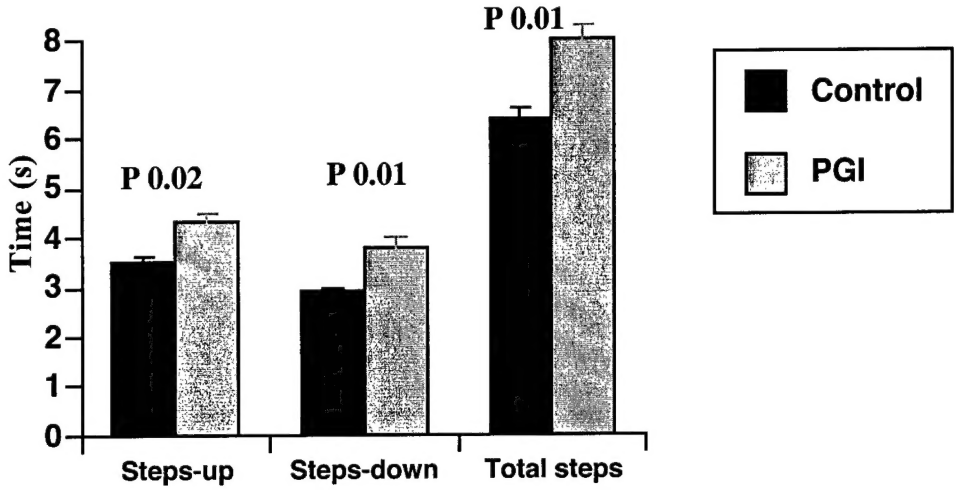

Fig. 3 Results of the timed stairclimbing tests in the healthy control veterans and the veterans with chronic fatigue (PGI).

31P-Magnetic Resonance Spectroscopy

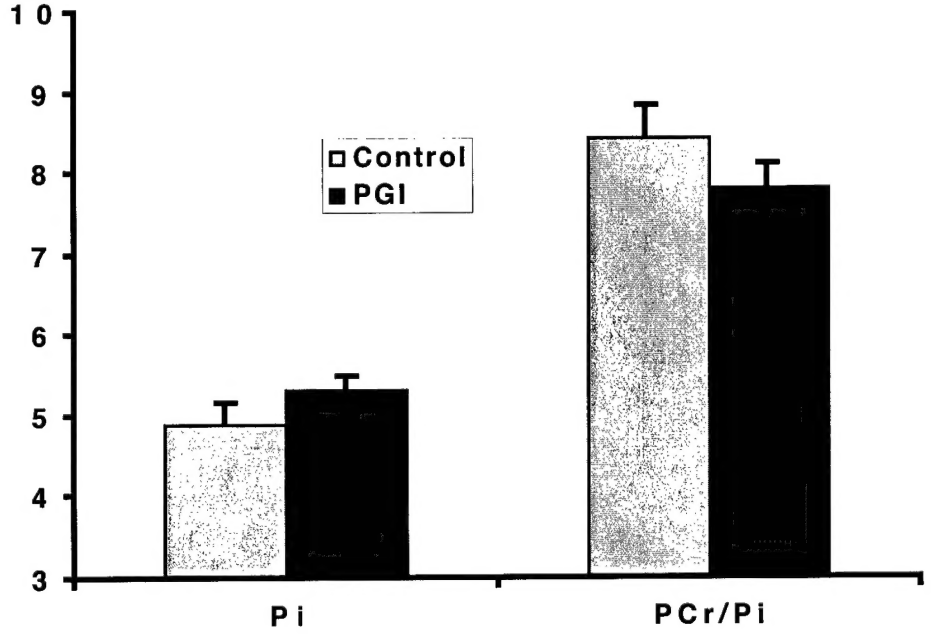

Fig. 4 Basal Pi concentration and PCr/Pi in the medial gastrocnemius of healthy control veterans and the veterans with chronic fatigue (PGI).

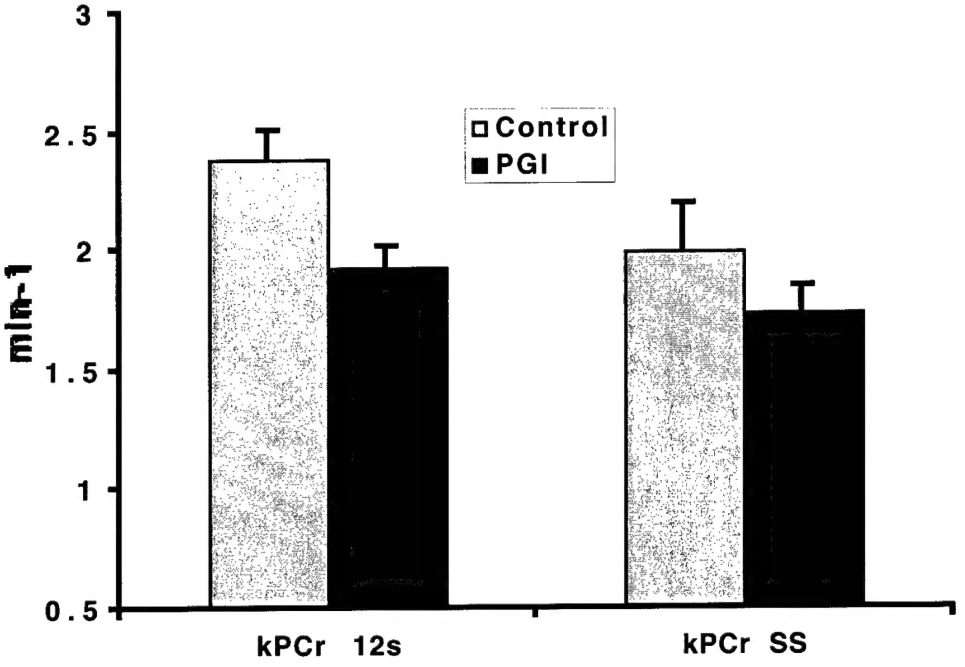

Fig. 5 PCr resynthesis rate (k_{PCr}) in the medial gastrocnemius of healthy control veterans and the veterans with chronic fatigue (PGI). kPCr 12s= PCr resynthesis rate following 12s "all-out" exercise; kPCr SS= PCr resynthesis rate following stady state exercise

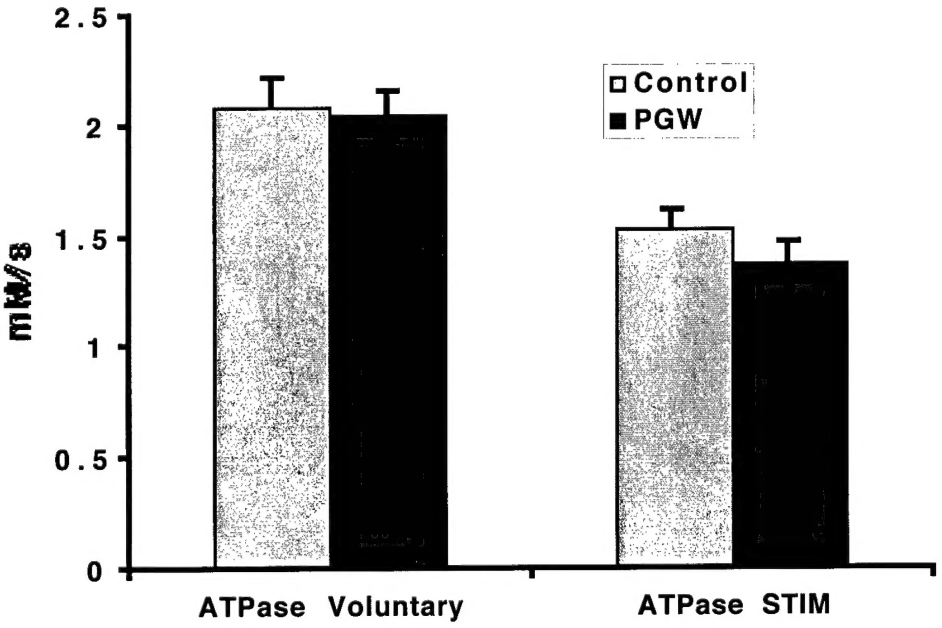

Fig. 6 ATPase rate during voluntary maximal exercise and electricall-induced maximal exercise in healthy control veterans and the veterans with chronic fatigue (PGI).

Biopsy results

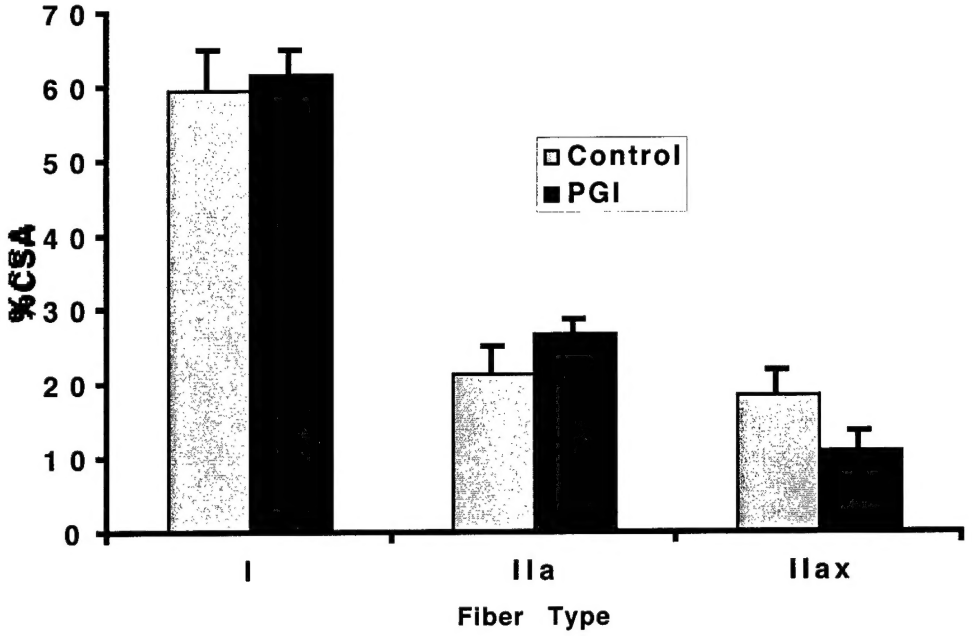

Fig. 7 Fiber type distribution of the medial gastrocenmius in healthy control veterans and veterans with chronic fatigue (PGI).

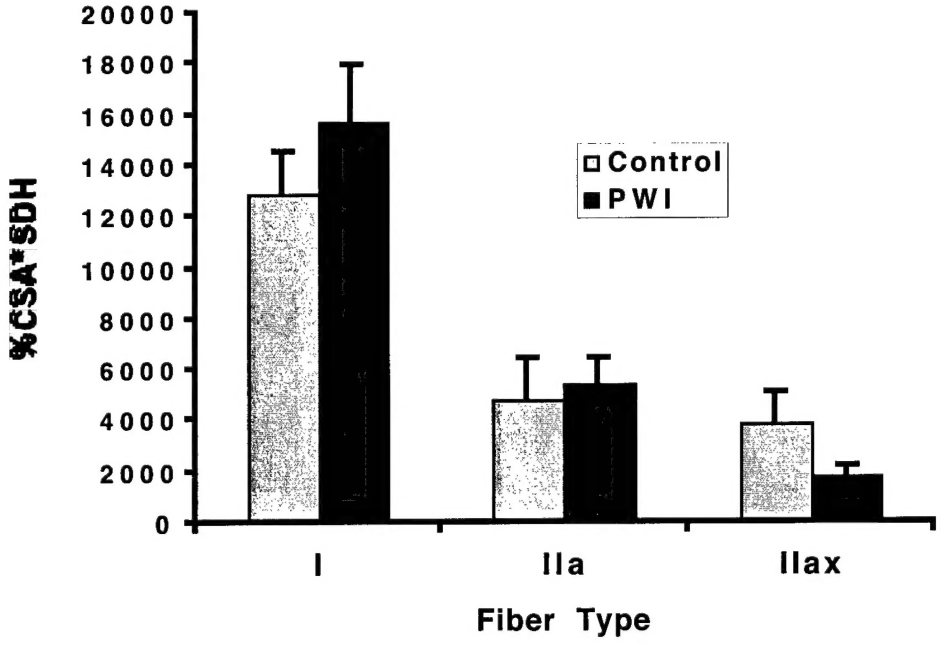

Fig. 8 Fiber type specific SDH in the medial gastrocenmius of healthy control veterans and veterans with chronic fatigue (PGI).

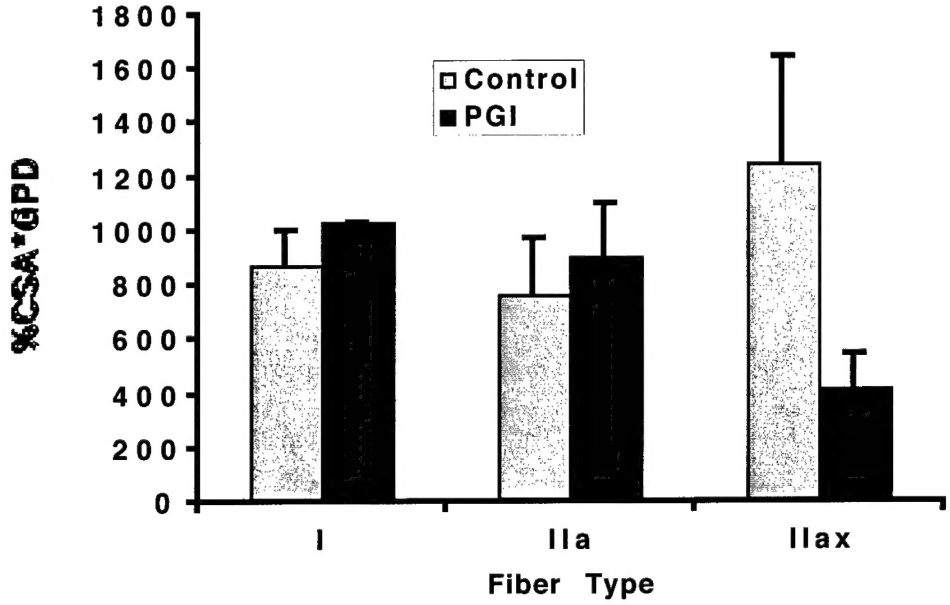

Fig. 9 Fiber type specific GPD in the medial gastrocenmius of healthy control veterans and veterans with chronic fatigue (PGI).

EMG results

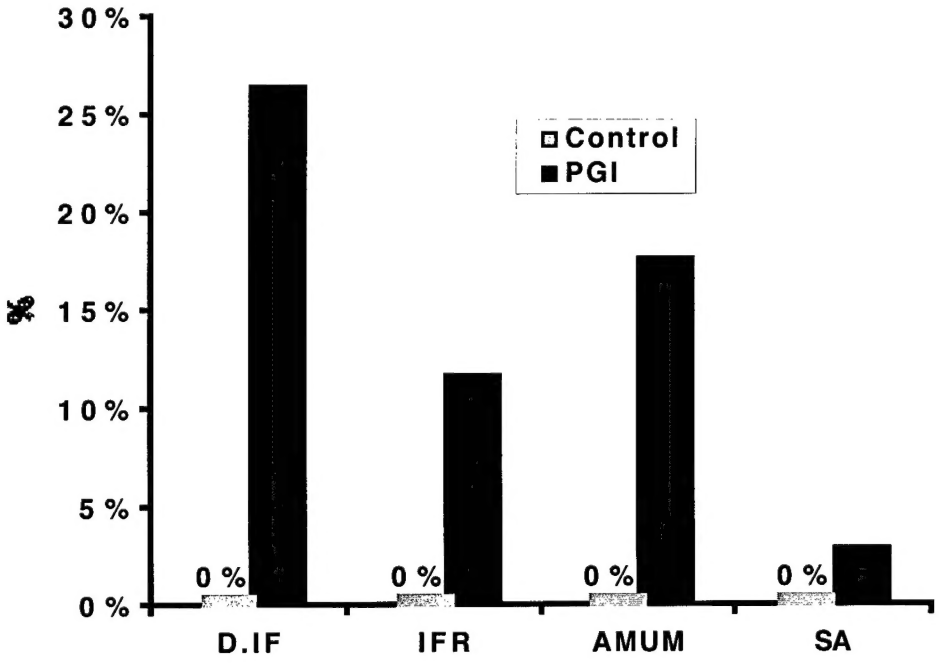

Fig. 10 Incidence of EMG abnormalities in healthy control veterans and veterans with chronic fatigue (PGI).